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Baculovirus Phosphoprotein pp31 Is Associated with Virogenic Stroma

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The *Pst*I K fragment of *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) encodes a protein with a molecular weight of 31,000. To define the role of this protein (pp31) in virus infection further, it was overexpressed in bacteria and used to produce polyclonal antiserum. Radioimmunoprecipitation analysis indicated that pp31 was synthesized during both the early and late phases of virus infection, consistent with previous analyses indicating that the gene was regulated by tandem early and late promoters. Metabolic labeling of cells with carrier-free phosphate indicated that pp31 was phosphorylated. Biochemical fractionation experiments showed that pp31 was localized in the nucleus and that it was more stably associated with the nucleus at later times of infection. Immunoblot analysis of subnuclear fractions indicated that pp31 was associated predominantly with the chromatin and nuclear matrix fractions. Immunofluorescence experiments confirmed that the pp31 protein was localized in the nucleus. Nuclear staining was relatively uniform early but was more centrally nuclear later in infection. Immunoelectron microscopy indicated that the pp31 protein was a component of virogenic stroma. Southwestern (DNA-protein) blot analysis demonstrated that pp31 is a DNA-binding protein. These findings suggest a possible role for pp31 in the virus life cycle.

In baculovirus-infected cells, viral genes are expressed in a temporally regulated fashion. Baculovirus genes have been classified as early, late, or very late, depending on their time of expression and their requirement for macromolecular synthesis. The early genes are further divided into two classes, immediate early and delayed early, based on results of transient expression assays. The immediate-early genes are highly expressed in the absence of other viral factors (8). The delayed-early genes require two immediate-early genes and a viral enhancer element for full expression (3, 9), although basal expression of some delayed-early genes can be detected in the absence of viral factors (12, 27). Expression of the late genes is coincident with the onset of viral DNA replication, while the very late genes are maximally expressed during the occlusion phase, after the peak of late-gene expression. At least two baculovirus genes, 39K and gp67, are expressed throughout infection and are regulated by tandem early and late promoters (2, 8).

Many of the baculovirus regulatory genes and elements were discovered by using a transient assay system based on the *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) 39K promoter (3, 8, 9, 19). As a result of these studies, considerable information on transcriptional regulation of this gene has accumulated. However, little is known concerning the function of the protein, pp31, encoded by this gene. The entire nucleotide sequence of the gene was recently determined (6). This sequence predicts a protein with a molecular weight of 31,280 that is rich in basic amino acids and has a predicted pI of 10.37. pp31 produced in rabbit reticulocyte extracts was shown to cross-react with an antiserum prepared against nuclear matrix fractions from infected cells (6, 29). However, this antiserum also cross-reacted with capsid, polyhedrin, and several unidentified proteins (unpublished data). To analyze the role of pp31 in infected cells further, the protein was expressed in bacteria and used to raise specific antiserum in rabbits. This antiserum was used to characterize the expression and intracellular localization of pp31.

MATERIALS AND METHODS

Cells and viruses. The conditions for Sf9 cell culture and infection with the E2 strain of Ac*M*NPV have been described previously (26). The virus AcP57VCβ is a polyhedrin-negative recombinant baculovirus that encodes a fusion protein containing the first 57 amino acids of polyhedrin fused to a large fragment of *Escherichia coli* β-galactosidase.

Production and characterization of polyclonal anti-pp31. A 1.9-kb *Pst*I-*Afl*II fragment of *Pst*I-K was cloned into the *Pst*I-*Hin*cII sites of mp18. Bacteriophage and plasmid DNAs were purified by the boiling procedure (11), precipitated with polyethylene glycol (13), and purified on cesium chloride-ethidium bromide gradients. Site-directed mutagenesis was performed by using the oligonucleotide TTACAAGAAGCA CATATGGTAAACGTG (20). Use of this oligonucleotide results in construction of an *Nde*I site (underlined) at the translation initiation codon of the pp31 (39K) protein-encoding gene. The desired mutant was cloned into T7 expression vector pET3a (22, 25). Cultures of DE3(*lysS*) recombinants were grown to an optical density at 600 nm of 0.6 and induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside. After 2 h at 37°C, cells were collected by centrifugation and suspended in sodium dodecyl sulfate (SDS) sample buffer (21), and the total cell lysates were electrophoresed on SDS–12% acrylamide gels. The proteins were visualized by staining with 0.1% Coomassie brilliant blue in water. After 5 min in stain, the gel was washed in water for 15 min. The 31K protein band was cut from the gel, lyophilized, and emulsified in phosphate-buffered saline.

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Two New Zealand White female rabbits were injected with approximately 500 μg of recombinant protein (10). After 1 month, the rabbits were given two injections 2 weeks apart. One week after the second injection, the rabbits were bled. Radioimmunoprecipitation was used to test for production of specific antiserum by using previously described methods (17). *Spodoptera frugiperda* cells were infected with Ac*M*NPV at a multiplicity of infection of 10 and labeled from 20 to 24 h postinfection with [^{35}S]Translabel, a mixture of methionine and cysteine. The labeled cells were treated with 1 ml of extraction buffer (50 mM Tris [pH 8.0], 100 mM NaCl, 1% Nonidet P-40) for 20 min on ice, and clarified by centrifugation. The resulting supernatant was incubated with 5 μl of preimmune rabbit serum or rabbit serum prepared against the pp31 protein. After 12 h at 4°C, the immune complexes were absorbed with 100 μl of a formalin-fixed *Staphlyococcus aureus* Cowan 1 suspension (18) and washed with extraction buffer supplemented with 0.1% SDS and 1% sodium deoxycholate. Washed immunoprecipitates were boiled in sample buffer for 3 min and clarified by centrifugation. Proteins were separated on an SDS–12% polyacrylamide gel (21). Commercially available ^{14}C-labeled marker proteins (Bethesda Research Laboratories) were used as size markers.

Metabolic labeling and biochemical fractionation. Cells were infected and labeled with [^{35}S]Translabel as described above or labeled with ^{32}P. After labeling, the cells were separated into cytosolic and nuclear fractions as previously described (15). Fractions were analyzed by radioimmunoprecipitation as described above or Western blotting (immunoblotting) as described below. Nuclear matrix fractionation was performed as previously described (24).

Western and Southwestern (DNA-protein) blot analyses. Samples for Western blot analysis were boiled for 3 min and electrophoresed on 12% acrylamide gels. The proteins were electrophoretically transferred to nitrocellulose sheets by using a semidry apparatus (10). The sheets were reacted with preimmune serum or pp31 antiserum essentially as previously described (17). Immune complexes were detected by using alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (IgG).

For Southwestern blots, 5×10^6 cells were seeded in a T-75 flask and mock infected or infected with Ac*M*NPV at a multiplicity of 10 PFU/ml. After 24 h, the cells were pelleted, washed once in phosphate-buffered saline, and suspended in 1 ml of extraction buffer. After 20 min of shaking on ice, the cells were microcentrifuged for 10 min. The supernatant was discarded, and the pellet containing the detergent-insoluble material was suspended in 0.5 ml of Laemmli sample buffer (21). A 20-μl sample was loaded onto SDS-polyacrylamide gels and electrophoresed. The proteins were electrophoretically transferred to nitrocellulose as described above. The nitrocellulose was cut into strips, and the proteins were renatured by three 1-h incubations in buffer A (10 mM Tris hydrochloride [pH 7.2], 5% skim milk, 10% glycerol, 2.5% Nonidet P-40, 0.1% dithiothreitol, 150 mM NaCl) at room temperature (14). The strips were washed briefly in buffer B (10 mM Tris hydrochloride [pH 7.2], 0.25% skim milk, 50 mM NaCl) and incubated in 10 ml of buffer B plus 1 μg of salmon sperm DNA per ml and 5×10^6 cpm of the indicated probe. Ac*M*NPV and lambda DNA were radiolabeled with [^{32}P]dATP by nick translation as previously described (23). The blots were hybridized overnight at 4°C with gentle shaking. The blots were rinsed twice in buffer B, dried, and exposed to film for 3 h. The amount of radioactivity bound to the filter was quantitated on a Betagen betascope.

Immunocytochemical localization of pp31. Sf9 cells were infected with Ac*M*NPV at a multiplicity of infection of about 10 PFU/cell or transfected with 20 μg of calf thymus or plasmid DNA. At various times postinfection or at 24 h posttransfection, the cells were fixed with formalin and permeabilized with methanol, and indirect immunofluorescence was performed as described previously (15). Plasmid pIE1-39K was constructed by digesting *Pst*I-K with *Bst*BI and *Afl*II. The 1,140-bp fragment containing the complete pp31-coding region was repaired with Klenow fragment and ligated into the *Hin*cII site of pIE1 located immediately downstream of the transcription start site for the IE1 gene (8).

Immunogold electron microscopy. *S. frugiperda* cells were infected with Ac*M*NPV at a multiplicity of 10. After 24 h, infected and uninfected control cells were fixed for 30 min at 4°C by using 1% paraformaldehyde and 0.5% glutaraldehyde in 0.05 M sodium cacodylate buffer at pH 7.1. The cells were dehydrated at ice bath temperature and infiltrated at −20°C with LR White with 95% ethanol as a diluent. After two changes of pure LR White, the cells were put into gelatin capsules and polymerized at −30°C by using long wavelength UV light. The LR White used for infiltration and embedding contained 1.5% (wt/vol) benzoin methyl ether (Polysciences, Warrington, Pa.) as a UV-active promoter.

Sections were cut with a diamond knife and picked up on Formvar-filmed, single-slot copper grids which had been lightly coated with carbon. The sections were incubated in the following reagents: Tris-buffered saline (TBS) for 15 min, TBS containing 5% (wt/vol) cold water fish gelatin (Sigma) for 15 to 30 min, primary antibody overnight at 4°C, TBS containing 0.05% (wt/vol) Tween 20 (three washes), TBS (three washes), goat anti-rabbit IgG coupled to 10-nm-diameter-gold particles (Amersham) for 30 min at a dilution of 1/20 in TBS, TBS containing Tween 20 (three washes). This was followed by three washes in double-distilled water. Control incubations were done by using the secondary IgG-gold only and preimmune IgG or an unassociated primary antibody followed by IgG-gold antibody. The IgG fractions of pp31 antiserum and control sera were purified on protein A-agarose as previously described (10).

Sections were viewed unstained or lightly stained with uranium and lead salts by using a 420 electron microscope (Philips, Mahwah, N.J.).

RESULTS

Immunodetection of pp31 in infected cells. A DNA fragment that encodes the 39K (pp31) protein was cloned into a T7 expression vector and expressed in *E. coli* as described in Materials and Methods. Crude bacterial cell lysates were fractionated on SDS-polyacrylamide gels, and the pp31 band was used to immunize rabbits (data not shown). The resulting polyclonal antiserum was used to analyze the synthesis of pp31 in Ac*M*NPV-infected cells. *S. frugiperda* cells were infected with wild-type virus, pulse-labeled for 4-h intervals at the indicated times postinfection and separated into nuclear and cytosolic fractions, and the fractions were immunoprecipitated as described in Materials and Methods (Fig. 1A). A viral protein with an apparent molecular weight of 33,000 was specifically precipitated in the first period (0 to 4 h postinfection). pp31 labeled during this period was equally distributed in the nuclear and cytosolic fractions. The peak of pp31 synthesis was from 4 to 8 h postinfection. During this labeling period, approximately 75% of the protein was localized in the nucleus. The rate of synthesis of pp31

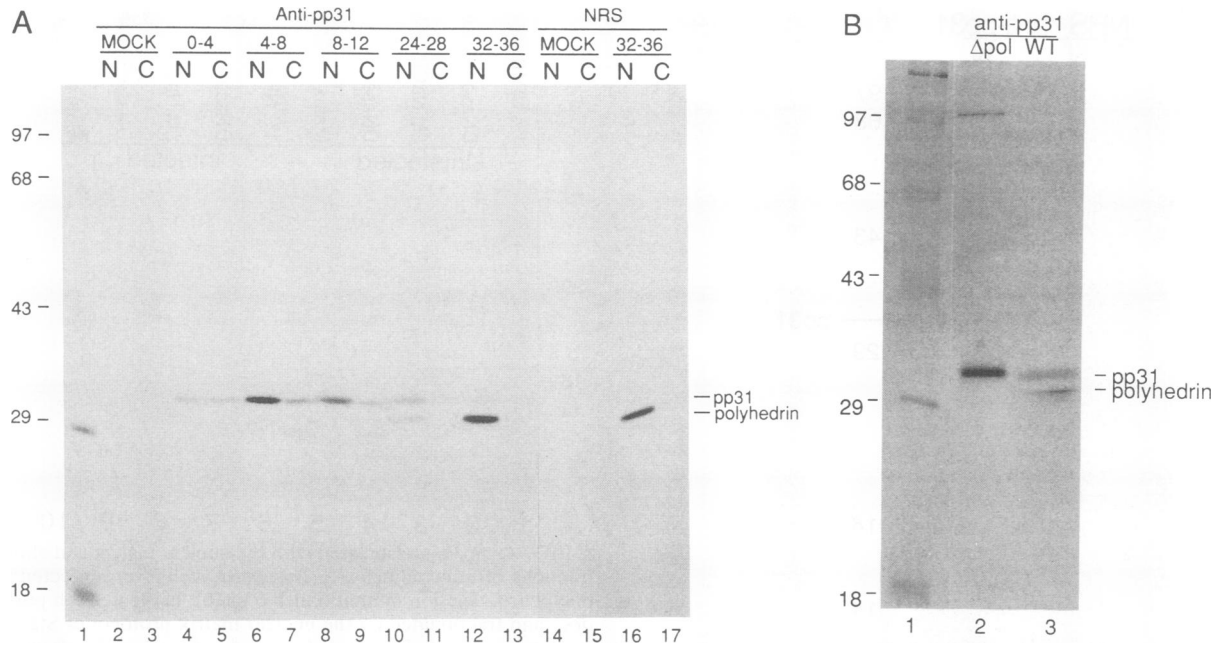

FIG. 1. Time course of pp31 synthesis in Ac*M*NPV-infected *S. frugiperda* cells. Ac*M*NPV-infected and mock-infected cells were metabolically labeled at the indicated times postinfection, harvested, and separated into nuclear (N) and cytoplasmic (C) fractions, and the fractions were immunoprecipitated by using the indicated antiserum. In both panels, the numbers at the left indicate the molecular sizes (in kilodaltons) of protein standards and the positions of the immunoreactive proteins are shown on the right. (A) Immunoprecipitation using pp31 antiserum (anti-pp31; lanes 2 to 13) or normal rabbit serum (NRS; lanes 14 to 17). (B) Radioimmunoprecipitation of pp31 from cells infected with wild-type virus (WT; lane 3) or a polyhedrin deletion mutant virus (Δpol; lane 2).

gradually decreased, and the relative amounts of pp31 found in the nuclear fraction increased as the infection cycle progressed.

At 24 h postinfection, a second protein with a molecular weight of approximately 31,000 was detected. This protein was also highly synthesized during the 32 to 36-h labeling period. The 31,000-molecular-weight protein appeared to be nonspecific, as it was also immunoprecipitated with normal rabbit serum. The size of this protein and the kinetics of its synthesis suggested that it might be polyhedrin. Polyhedrin often immunoprecipitates nonspecifically, owing to its inherent insolubility. To test this possibility, *S. frugiperda* cells were infected with wild-type virus and a polyhedrin deletion mutant (AcP57VCβ). Nuclear extracts were prepared at 36 h postinfection and immunoprecipitated with pp31 antiserum (Fig. 1B). The 33,000-molecular-weight protein was detected in extracts from cells infected with either virus, while the 31,000-molecular-weight protein was detected only in the extract prepared from wild-type virus-infected cells, indicating that this protein was polyhedrin. Comparison of the amino acid sequences of polyhedrin and pp31 did not reveal significant sequence similarity, indicating that the reactivity was not sequence specific (data not shown).

Accumulation of pp31 in infected cells was analyzed by immunoblot analysis of nuclear and cytosolic fractions isolated at the indicated times postinfection. Examination of the immunoblot revealed that pp31 was first detectable at 8 h postinfection in the nuclear fraction (Fig. 2A). Protein accumulation continued through 36 h and declined slightly after that. Relatively little pp31 was detectable in the cytosolic fractions at any time. A protein with an apparent molecular weight of 31,000 was first observed at 28 h postinfection. This protein was not detected in cells infected with polyhe-

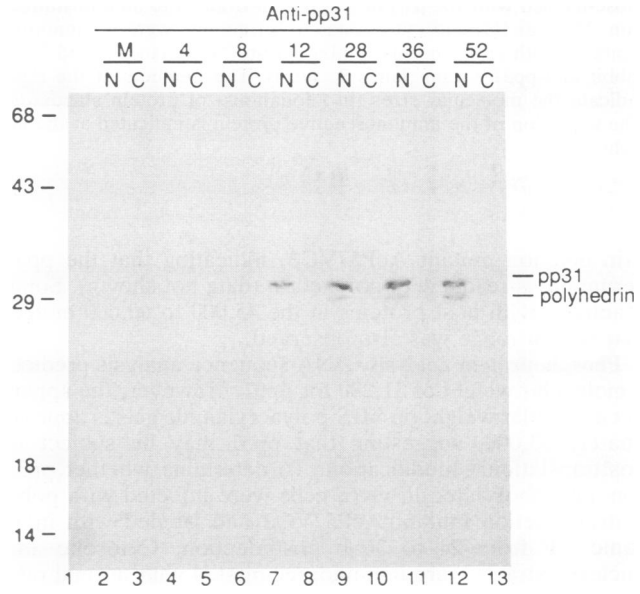

FIG. 2. Immunoblot analysis of pp31 synthesized in infected cells. Nuclear (N) and cytosolic (C) fractions were prepared from mock-infected cells (M) or from infected cells at the indicated times postinfection, separated on SDS–12% polyacrylamide gels, transferred to nitrocellulose membranes, and probed with pp31 antiserum. The numbers at the left indicate the molecular sizes (in kilodaltons) of protein standards. The numbers at the right indicate the positions of the immunoreactive proteins.

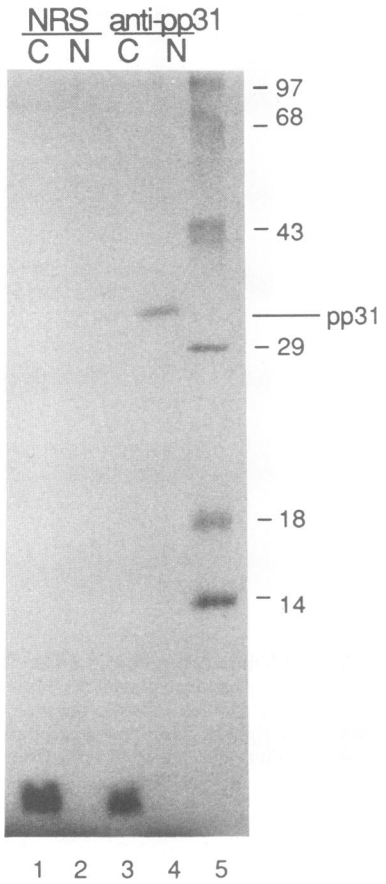

FIG. 3. Phosphoprotein analysis of pp31. *S. frugiperda* cells were infected with a polyhedrin deletion mutant of Ac*M*NPV and pulse-labeled with 100 μCi of $^{32}P_i$ per ml from 24 to 36 h postinfection. Nuclear (N) and cytoplasmic (C) fractions were immunoprecipitated with either normal rabbit serum (NRS; lanes 1 and 2) or rabbit anti-pp31 serum (lanes 3 and 4). The numbers at the right indicate the molecular sizes (in kilodaltons) of protein standards. The migration of the immunoreactive protein is indicated at the far right.

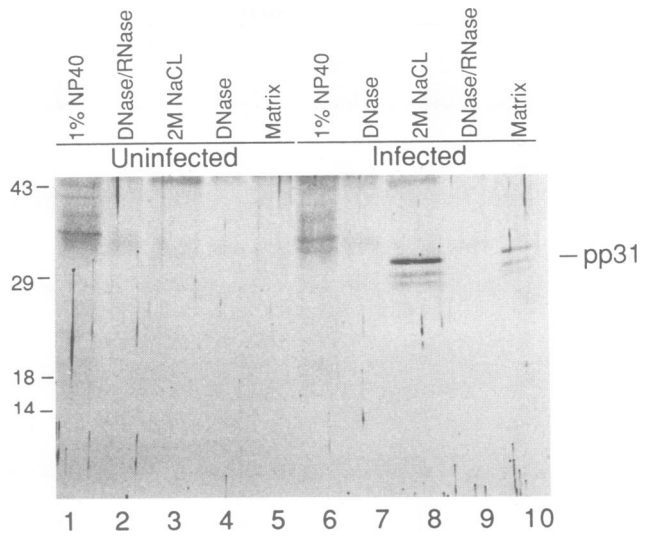

FIG. 4. pp31 is associated with the nuclear matrix and chromatin fractions of infected cells. *S. frugiperda* cells were infected with a polyhedrin deletion mutant (lanes 6 to 10), harvested 8 h postinfection, and fractionated by the nuclear matrix protocol of Staufenbiel and Deppert (24). Uninfected control cells were also fractionated (lanes 1 to 5). Aliquots of the fractions were separated on SDS-polyacrylamide gels, transferred to nitrocellulose, and probed with pp31 antiserum. Lanes: 1 and 6, 1% Nonidet P-40-soluble fraction; 2 and 7, material released by treatment with DNase I and RNase; 3 and 8, proteins released by treatment with 2 M NaCl (chromatin fraction); 4 and 9, proteins released by DNase I following extraction by a high salt concentration; 5 and 10, proteins remaining in the insoluble fraction following all treatments. The numbers on the left are molecular sizes in kilodaltons.

drin deletion mutant AcP57VCβ, indicating that the pp31 serum cross-reacts with polyhedrin (data not shown). Some reactivity with host proteins in the 45,000 to 60,000 molecular weight range was also observed.

Phosphoprotein analysis. DNA sequence analysis predicts a molecular weight of 31,280 for pp31. However, the apparent molecular weight on SDS-polyacrylamide gels is approximately 33,000, suggesting that pp31 may be subject to posttranslational modification. To determine whether pp31 was phosphorylated in vivo, cells were infected with polyhedrin deletion mutant AcP57VCβ and labeled with inorganic ^{32}P from 24 to 36 h postinfection. Cytosolic and nuclear extracts were immunoprecipitated with normal rabbit serum or anti-pp31 serum and analyzed on SDS-polyacrylamide gels (Fig. 3). The results of this analysis indicated that a 33,000-molecular-weight protein that specifically reacts with anti-pp31 was labeled with ^{32}P. As shown above for ^{35}S-labeled pp31, most of the ^{32}P-radiolabeled protein was detected in the nuclear fraction. Essentially identical results were obtained with cells labeled from 12 to 24 h postinfection (data not shown). These data indicate that pp31 is phosphorylated. The low-molecular-weight band in the cytoplasmic fraction was probably phospholipid.

Nuclear matrix fractionation. Previous results have demonstrated that pp31 reacted with an antiserum prepared against a complex of proteins that copurified with nuclear matrix proteins from infected cells (6, 29). However, this antiserum also cross-reacted with polyhedrin, capsid, and other, unidentified proteins (data not shown). To confirm that pp31 is actually a component of the nuclear matrix, infected and control cells were fractionated by using an established in situ procedure (24). Aliquots of the proteins solubilized at each step were separated on SDS-polyacrylamide gels, transferred to nitrocellulose, and probed with anti-pp31 serum (Fig. 4). This analysis revealed that pp31 was partially solubilized from nuclei with 2 M NaCl, a treatment which releases chromatin-bound proteins (lane 8). The remaining pp31 was stably associated with the nuclear matrix fraction (lane 10).

Indirect immunofluorescence. Another approach used to determine the intracellular distribution of pp31 was indirect immunofluorescence. Sf9 cells were infected with Ac*M*NPV and examined by indirect immunofluorescence at various times postinfection (Fig. 5). At relatively early times postinfection (8 h; Fig. 5B), Sf9 nuclei were uniformly stained by anti-pp31, and this reaction appeared to be specific, as normal rabbit serum did not react with these cells (Fig. 5A). Later, the reactivity was more centrally localized within the nucleus (24 h in Fig. 5C, 48 h in Fig. 5D). Sf9 cells were transfected with a plasmid that expresses pp31 under control of the IE1 promoter. These cells also exhibited uniform

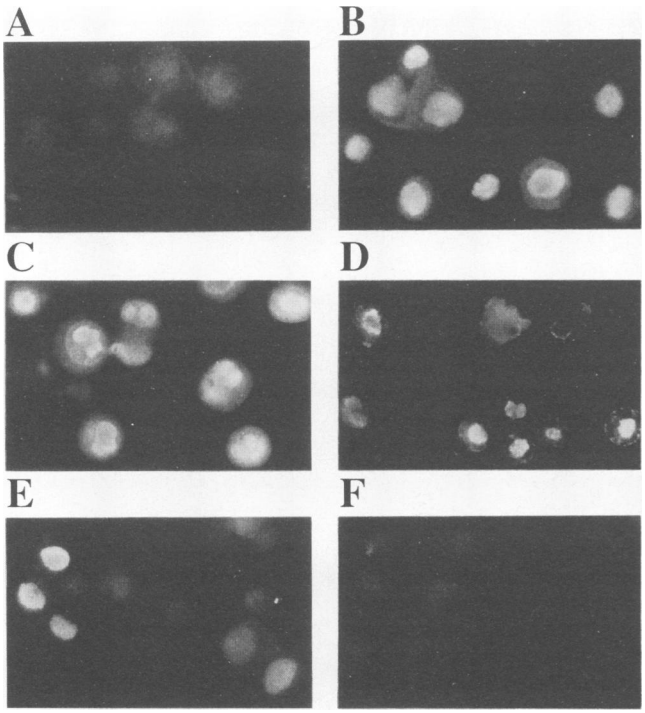

FIG. 5. Intracellular distribution of pp31 by indirect immunofluorescence. Sf9 cells were infected with Ac*M*NPV and stained with anti-pp31 at 8 (B), 24 (C), or 48 (D) h postinfection as described in Materials and Methods. Panel A shows a control in which the cells were infected for 24 h and stained with normal rabbit serum. Panels E and F show cells transfected with pIE1-39K and stained at 24 h posttransfection with either anti-pp31 (E) or normal rabbit serum (F).

nuclear staining with anti-pp31 (Fig. 5E) but not with normal rabbit serum, supporting the idea that the nuclear reactivity is specific (Fig. 5F). This result also indicates that no other viral functions are required for nuclear localization of pp31.

Immunogold staining of pp31. To extend the immunofluorescence results, immunogold staining of pp31 in ultrathin sections of Ac*M*NPV-infected cells was performed. Consistent with the indirect immunofluorescence data, anti-pp31 specifically stained a virogenic stroma at 24 h postinfection (Fig. 6A). Within the stroma, the gold particles were concentrated over the condensed nucleic acid fibrils. There was no specific staining of the space between the electron-dense filaments or of nucleocapsids in the stroma. There was also no staining of the enveloped nucleocapsid bundles that assemble in the nucleus in the zone surrounding the stroma. IgGs purified from normal rabbit serum were tested at the same dilution and did not specifically stain the virogenic stroma (Fig. 6B). Both control and pp31 IgGs reacted nonspecifically with cytoplasmic vacuoles in infected and uninfected cells (data not shown).

Southwestern blots. The presence of pp31 in the condensed nucleic acid region of a virogenic stroma suggested that pp31 functions as a DNA-binding protein. Southwestern analysis was performed by using radiolabeled DNA probes to determine whether pp31 binds to viral DNA. *S. frugiperda* cells were mock infected or infected with Ac*M*NPV. After 24 h, nuclear extracts were prepared and the proteins were separated on SDS-polyacrylamide gels (Fig. 7). After transfer to nitrocellulose, the proteins were renatured and probed with nick-translated Ac*M*NPV or lambda DNA. In uninfected cells, five proteins were detected with Ac*M*NPV DNA as a probe (lane 4). The same proteins were also present in Ac*M*NPV-infected cells. In addition, a virus-specific protein also bound viral DNA (lane 5). This protein comigrated with the protein that cross-reacted with pp31 antiserum (lanes 2 and 3). An identical pattern was observed with labeled lambda DNA (lanes 6 and 7). Quantitation of the radioactivity bound to pp31 revealed that the two probes bound equally, indicating that pp31 is a nonspecific DNA-binding protein. pp31 expressed in bacteria also showed non-sequence-specific DNA-binding activity (data not shown).

DISCUSSION

To characterize the function of the Ac*M*NPV 39K gene product further, pp31 was expressed in bacteria, gel purified, and injected into rabbits. Antibodies raised against pp31 were used to characterize the expression, cellular localization, and biochemical properties of this viral protein. Radioimmunoprecipitation analysis of extracts from radiolabeled cells showed that pp31 was expressed during both the early and late phases of viral infection. This is consistent with our previous results, which indicate that this gene is regulated by tandem early and late promoters and is highly transcribed during both phases of viral replication (7, 8).

Biochemical fractionation experiments showed that pp31 is predominantly nuclear in Ac*M*NPV-infected cells. At earlier times of infection, relatively higher amounts of cytosolic pp31 were observed by pulse-labeling and radioimmunoprecipitations. However, immunoblotting showed that minimal amounts of pp31 accumulated in the cytosolic fraction. These results suggest that newly synthesized pp31 is relatively loosely associated with the nucleus at earlier times of infection and can be extracted into the cytosolic fraction during the biochemical fractionation procedure. In support of this interpretation, pp31 appeared to be exclusively nuclear by indirect immunofluorescence. Furthermore, the immunofluorescence staining pattern was different at early and later times of infection. Earlier in infection pp31 appears to be uniformly distributed throughout the nucleus, but later it becomes more centrally located. This probably reflects the incorporation of pp31 into the virogenic stroma (see below), which could account for the relatively stronger association of pp31 with the nucleus later in infection. Nuclear localization of pp31, per se, does not require any other viral functions, as transiently expressed pp31 had the same intranuclear distribution as was observed at early times in infected cells by indirect immunofluorescence. We have observed similar results for the Ac*M*NPV polyhedrin protein (16).

Immunoelectron microscopy showed the intranuclear distribution of pp31 in more detail and indicated that it accumulates in the virogenic stroma. The virogenic stroma is the predominant structure in the nucleus from 8 to 48 h postinfection (5). The virogenic stroma is a chromatin-like network of electron-dense filaments. Ultrastructure studies have demonstrated that packaging of virus particles occurs in the stroma. Empty viral capsids assemble in the pockets between filaments, and then the capsids fill with DNA acquired from the stroma (4). The virogenic stroma is also believed to be the site of viral DNA replication and transcription, although this has not been directly demonstrated. The presence of pp31 in the condensed nucleic acid region of the stroma is consistent with a role in replication or transcription. pp31 may also be involved in preparing the DNA for

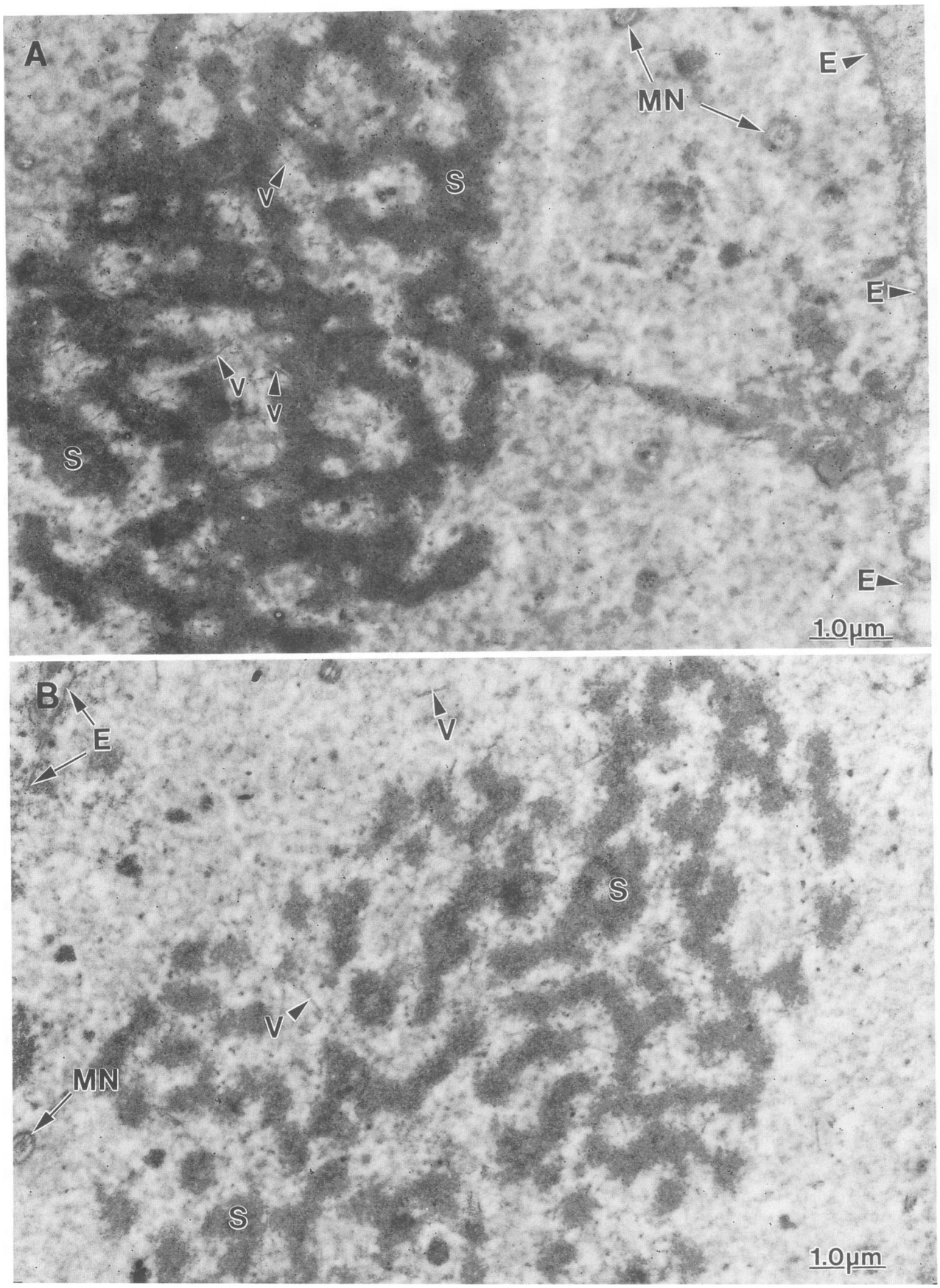

FIG. 6. Ultrastructural localization of pp31 in Ac*M*NPV-infected *S. frugiperda* cells at 24 h postinfection. (A) Cells stained with pp31 antiserum showing immunogold labeling in the electron-dense region of the virogenic stroma (S). Single nucleocapsids (V) within the stroma and bundles of nucleocapsids surrounded by a common envelope (MN) are also indicated. The nuclear envelope (E) is indicated. (B) Cells treated with control serum show no specific staining in the stroma region.

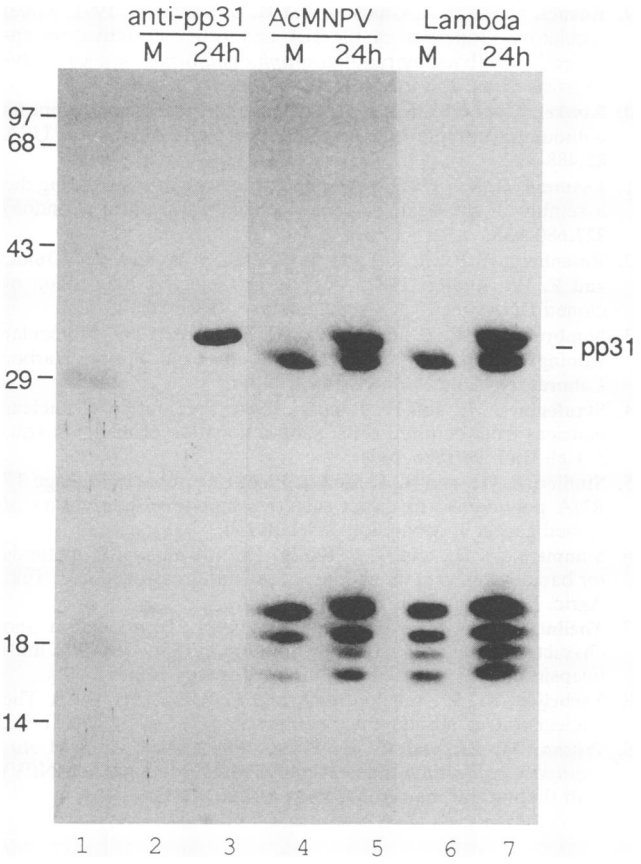

FIG. 7. Southwestern blot analysis of DNA-binding proteins isolated from uninfected and infected cells. *S. frugiperda* cells were mock infected (M; lanes 2, 4, and 6) or infected with wild-type Ac*M*NPV (24h; lanes 3, 5, and 7). After 24 h, nuclear extracts were prepared and fractionated on SDS-polyacrylamide gels. After transfer to nitrocellulose, proteins were incubated with pp31 antiserum (lanes 2 and 3) or renatured and probed with ^{32}P-labeled Ac*M*NPV DNA (lanes 4 and 5) or ^{32}P-labeled lambda DNA (lanes 6 and 7). The molecular sizes of marker proteins (lane 1) are indicated in kilodaltons on the left. The position of the immunoreactive protein is indicated on the right.

packaging, although it does not appear to be a viral structural protein. Western blot analysis of purified extracellular virus did not reveal the presence of pp31 in the virus (data not shown). This is consistent with the finding that virus particles were not stained with gold particles.

The nuclear matrix is an ill-defined structure. It consists of the cellular material remaining after extraction with nonionic detergent, DNase, RNase, and a high salt concentration. Structural studies indicate that the matrix is composed of lamina with associated pore complexes, nucleoli, and intranuclear material. In addition to its structural importance, the matrix may also be involved in diverse cellular functions. Both DNA replication and transcription are linked to the nuclear matrix (for reviews, see references 1 and 28). Analysis of cells infected with adenovirus, herpesvirus, or polyomaviruses have revealed an enrichment of virus-specific proteins in nuclear matrix preparations (1, 28). The virogenic stroma is a unique structure that occurs only in baculovirus-infected cells (4, 5). Biochemical fractionation experiments revealed that pp31 is associated with chromatin and nuclear matrix fractions, while immunoelectron microscopy showed that pp31 was localized to the nucleic acid material component of the virogenic stroma. Thus, these data suggest that the virogenic stroma is a specialized form of the nuclear matrix which presumably serves the same function as the nuclear matrix.

pp31 is a DNA-binding protein, although it does not appear to be sequence specific, at least with the assay used here. Southwestern blot analysis indicated that Ac*M*NPV and lambda DNAs bound to pp31 with the same efficiency. The DNA sequences that tightly associate with the nuclear matrix after a combination of nuclease digestion and extraction with a high salt concentration have been analyzed. These sequences, called matrix attachment regions, are not strictly conserved but are typically 70% A+T (1). It will be of interest to determine whether the Ac*M*NPV genome contains sequences equivalent to matrix attachment regions. This may allow us to design a better assay to test whether pp31 preferentially binds specific viral sequences.

The pp31 protein interacts both with the nuclear framework and with DNA, suggesting that its role in viral replication is to form a structural lattice for the organization of viral DNA. Our attempts to construct a viral mutant with deletions in the pp31-encoding gene have proved unsuccessful (5a). While not definitive, this result is consistent with our hypothesis that pp31 plays an essential role in the virus life cycle. Confirmation of this hypothesis requires further analysis.

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REFERENCES

1. **Berezny, R.** 1991. The nuclear matrix: a heuristic model for investigating genomic organization and function in the cell nucleus. J. Cell. Biochem. **47:**109–123.
2. **Blissard, G. W., and G. F. Rohrmann.** 1989. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orygia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology **168:**354–362.
3. **Carson, D. D., L. A. Guarino, and M. D. Summers.** 1988. Functional mapping of an AcMNPV immediate early gene which augments IE-1 transactivation of the 39K gene. Virology **162:**444–451.
4. **Fraser, M. J.** 1986. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected Spodoptera frugiperda cells. J. Utrastruct. Mol. Struct. Res. **95:**189–195.
5. **Granados, R. R., and L. A. Lawler.** 1981. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology **108:**297–308.

5a. **Guarino, L. A., and D. L. Jarvis.** Unpublished data.

6. **Guarino, L. A., and M. W. Smith.** 1990. Nucleotide sequence of the 39K gene region of Autographa californica nuclear polyhedrosis virus. Virology **179:**1–8.
7. **Guarino, L. A., and M. W. Smith.** 1992. Regulation of delayed early gene transcription by dual TATA boxes. J. Virol. **66:**3733–3739.
8. **Guarino, L. A., and M. D. Summers.** 1986. Functional mapping of a *trans*-activating gene required for expression of a baculovirus delayed-early gene. J. Virol. **57:**563–571.
9. **Guarino, L. A., and M. D. Summers.** 1986. Interspersed homol-

ogous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J. Virol. **60:**215–223.
10. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:**193–197.
12. **Hoopes, R. R., and G. F. Rohrmann.** 1991. In vitro transcription of baculovirus immediate early genes: accurate mRNA initiation by nuclear extracts from both insect and human cells. Proc. Natl. Acad. Sci. USA **88:**4513–4517.
13. **Humphreys, G. O., G. A. Willshaw, and E. A. Anderson.** 1975. A simple method for the precipitation of large quantities of pure plasmid DNA. Biochim. Biophys. Acta **383:**457–463.
14. **Imbalzano, A. N., A. A. Shepard, and N. A. DeLuca.** 1990. Functional relevance of specific interactions between herpes simplex virus type 1 ICP4 and sequences from the promoter-regulatory domain of the viral thymidine kinase gene. J. Virol. **64:**2620–2631.
15. **Jarvis, D. L., D. A. Bohlmeyer, and A. Garcia, Jr.** 1991. Requirements for nuclear localization and supramolecular assembly of a baculovirus polyhedrin protein. Virology **185:**795–810.
16. **Jarvis, D. L., D. A. Bohlmeyer, and A. Garcia, Jr.** 1992. Enhancement of polyhedrin nuclear localization during baculovirus infection. J. Virol. **66:**6903–6911.
17. **Jarvis, D. L., and M. D. Summers.** 1989. Glycosylation and secretion of human tissue plasminogen activator in recombinant baculovirus-infected insect cells. Mol. Cell. Biol. **9:**214–223.
18. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115:**1617–1624.
19. **Kovacs, G. R., L. A. Guarino, and M. D. Summers.** 1991. Novel regulatory properties of the IE1 and IE0 transactivators encoded by the baculovirus *Autographa californica* nuclear polyhedrosis virus. J. Virol. **65:**5281–5288.
20. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic expression. Proc. Natl. Acad. Sci. USA **82:**488–492.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
22. **Rosenberg, A. H., B. N. Lade, D. S. Chui, S. W. Lin, J. J. Dunn, and F. W. Studier.** 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene **56:**125–135.
23. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual; 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Staufenbiel, M., and W. Deppert.** 1984. Preparation of nuclear matrices from cultured cells: subfractionation of nuclei in situ. J. Cell Biol. **98:**1886–1894.
25. **Studier, F. W., and B. A. Moffat.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189:**113–130.
26. **Summers, M. D., and G. E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. Tex. Agric. Exp. Stn. Bull. **1555.**
27. **Theilmann, D. A., and S. Stewart.** 1991. Identification and characterization of the IE1 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology **180:**492–508.
28. **Verheijen, R., W. van Venrooij, and F. Ramaekers.** 1988. The nuclear matrix: structure and composition. J. Cell Sci. **90:**11–36.
29. **Wilson, M. E., and K. H. Price.** 1988. Association of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) with the nuclear matrix. Virology **167:**233–241.